คุณภาพทางจุลชีววิทยาของน้ำเสียหลังผ่านการกำจัดฤทธิ์ของยาปฏิชีวนะโดยการใช้ไฮโดรเจนเปอร์ออกไซด์ที่อุณหภูมิสูง

MICROBIOLOGICAL EVALUATION OF COMBINED HYDROGEN PEROXIDE AND HEAT TREATMENT ON ANTIBIOTIC WASTEWATER

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บทคัดย่อ

การปล่อยยาปฏิชีวนะไปสู่สิ่งแวดล้อมอาจนำไปสู่การเสียสมดุลอย่างรุนแรงของระบบนิเวศ หนึ่งในเทคโนโลยีแบบใหม่สำหรับการบำบัดน้ำเสียที่ปนเปื้อนยาปฏิชีวนะ ได้แก่ การประยุกต์ใช้กระบวนการออกซิเดชั่นขั้นสูง งานวิจัยนี้มีวัตถุประสงค์เพื่อสาธิตการใช้ไฮโดรเจนเปอร์ออกไซด์ร่วมกับการใช้อุณหภูมิสูงที่ระดับต่าง ๆ ในการยับยั้งฤทธิ์ของยาปฏิชีวนะ

ทั้งนี้ยาปฏิชีวนะที่ใช้ศึกษามี 3 ชนิด ได้แก่ เซฟตาซิดิม เซฟไตรอะโซน และเซฟาเลกซิน ในระบบน้ำเสียจำลองความเข้มข้นเฉลี่ยของน้ำล้างที่จัดเป็นน้ำเสียได้จากการทำความสะอาดเครื่องจักรในกระบวนการผลิตโดยมีความเข้มข้นประมาณ 10 ไมโครกรัมต่อมิลลิลิตร แบบจำลองน้ำเสียที่มีการปนเปื้อนของยาปฏิชีวนะถูกเตรียมที่ 60 ไมโครกรัม/มิลลิลิตรของแต่ละยาปฏิชีวนะที่ศึกษา และทำการวิเคราะห์สารตกค้างด้วยเครื่องโครมาโทกราฟีของเหลวสมรรถนะสูง โดยทำการทดสอบกระบวนการออกซิเดชั่นขั้นสูงที่ความเข้มข้นไฮโดรเจนเปอร์ออกไซด์ร้อยละ 1 3 และ 5 โดยปริมาตร และที่ระดับอุณหภูมิ 60 80 และ 100 องศาเซลเซียส ที่เวลาต่าง ๆ ทั้งนี้มีการเติมเชื้ออีโคไลปริมาณ 6 ล็อคโคโลนีต่อมิลลิลิตร ลงในน้ำเสียที่จะบำบัดเพื่อเป็นตัวบ่งชี้ประสิทธิภาพของกระบวนการออกซิเดชั่น

ขั้นสูง ไฮโดรเจนเปอร์ออกไซด์ที่เหลืออยู่ในตัวอย่างหลังการบำบัดด้วยกระบวนการออกซิเดชั่นมีการกำจัดด้วยการเติมยีสต์ผง

ผลการวิจัยพบว่า ที่อุณหภูมิสูงขึ้น ความเข้มข้นของไฮโดรเจนเปอร์ออกไซด์มากขึ้นและใช้เวลาในการบำบัดนานขึ้นพบว่า มีประสิทธิภาพที่ดีในการกำจัดยาปฏิชีวนะตกค้าง ปริมาณของยาปฏิชีวนะตกค้างหลังจากกระบวนการออกซิเดชั่นแต่งต่างกันขึ้นอยู่กับระดับความสามารถในการกำจัดยาปฏิชีวนะแต่ละตัว ซึ่งการกำจัดเซฟตาซิดิม ทำได้ดีที่สุด รองลงมาคือเซฟไตรอะโซนและเซฟาเลกซิน ตามลำดับ ที่อุณหภูมิ 100 องศาเซลเซียส ความเข้มข้นของไฮโดรเจนเปอร์ออกไซด์ร้อยละ 5 ใช้เวลา 30 นาที สามารถกำจัดยาปฏิชีวนะได้สมบูรณ์ การใช้ไฮโดรเจนเปอร์ออกไซด์ร้อยละ 1 และ 3 พบว่าใช้เวลาบำบัดนานขึ้นเป็น 60 และ 120 นาที ตามลำดับ เงื่อนไขสภาวะออกซิเดชั่นที่อุณหภูมิ 100 องศาเซลเซียส สามารถที่จะกำจัดสารตกค้างที่มีการปนเปื้อนของเซฟไตรอะโซนและเซฟาเลกซิน ไฮดรอกซิลเรดิคัลที่เกิดขึ้นสามารถเร่งอัตราการทำลายการปนเปื้อนของยาปฏิชีวนะในน้ำเสีย

การใช้ไฮโดรเจนเปอร์ออกไซด์ร่วมกับอุณหภูมิประสบความสำเร็จในการนำมาประยุกต์ใช้ทำลายฤทธิ์ของยาปฏิชีวนะที่ปนเปื้อนในน้ำเสีย โดยที่ไม่ก่อให้เกิดสารประกอบที่ไม่เป็นอันตราย สามารถสูญสลายได้ง่าย ไฮดรอกซิลเรดิคัลที่เกิดขึ้นในระหว่างกระบวนการถูกสมมติฐานว่ามีความสามารถในการออกซิเดชั่นที่สูงในการบำบัดน้ำเสียด้วยไฮโดรเจนเปอร์ออกไซด์

คำสำคัญ: การปนเปื้อนของยาปฏิชีวนะในน้ำเสีย การใช้ความร้อน ไฮโดรเจนเปอร์ออกไซด์ การประเมินทางจุลชีววิทยา

Abstract

The release of trace pharmaceutical antibiotics into the environment can cause a major upset of an ecological balance. One of the promising technologies for treating antibiotic wastewater is the application of advanced oxidation processes.

Method: Hydrogen peroxide (H_2O_2) treatment was proposed as a pretreatment to remove ceftazidime, ceftriaxone, and cephalexin contaminants in a model of antibiotic wastewater. An averaged concentration of antibiotic contained in the first washed wastewater obtained from the major cleaning at the end of production was determined at approximately 10 μg/mL. The model wastewater of antibiotic production was formulated at 60 μg/mL of each antibiotics for safety reason and practical aspect of High Performance Liquid Chromatography (HPLC) analysis. H_2O_2 concentrations were varied at 1 3 and 5% (w/v) and the incubation temperatures were set at 60 80 and 100 °C. *E. coli* culture at log 6 CFU/mL initial density were added to the treated wastewater to evaluate the remaining antibiotic toxicity and assess the biocidal and biostatic effects. The inhibitory effect of H_2O_2 residues at the end of H_2O_2 treatment was neutralized successfully by adding dried bakers' yeast to catalyze oxygen and water conversions.

Result: Higher temperature, higher hydrogen peroxide concentration and longer hydrogen peroxide treatment time were the most effective to degrade antibiotic pollutants. The measurement of trace antibiotic at the end of H_2O_2 treatment suggested the different degree of degradation recalcitrance following this order ceftazidime was provided better degradation than ceftriaxone and cephalexin respectively. At 100°C, complete removal of antibiotics of 5% H_2O_2 treatment was achieved within 30 min. Longer duration was required in the case of 1 and 3% H_2O_2 treatment at 60 and 120 min, respectively. Strong oxidation condition (100 °C and 5%

H_2O_2) enabled instant removals of ceftriaxone and cephalexin. Hydroxyl radicals (•OH) was assumed to accelerate the fast degradation rate of antibiotic contaminants.

Conclusion: Combined hydrogen peroxide and heat treatment has been successfully applied for the degradation of antibiotic wastewater, either to less harmful compounds or to their complete mineralization. The hydroxyl radical's availability was hypothesized to provide strong oxidation potency of this successful H_2O_2 treatment scheme.

Keywords: Antibiotic Wastewater, Heat Treatment, Hydrogen Peroxide, Microbiological Evaluation

Introduction

In modern pharmacy, antibiotics are specifically designed as the inhibitory controllers of their respective pathogenic bacteria both in human and animals. Their major benefits included health prevention, therapeutic treatment from infectious illnesses in most organisms, as well as growth supplements in veterinary industry such as livestock, marine and agricultural farming [1-2]. Pharmaceutical consumption and production of antibiotics were continuously increasing throughout the world especially in industrialized and developing countries. Unfortunately, there were frequent detections of their residues releasing from industrial wastewater and secretion of human and animals into water systems [3-4]. The extensive usage of antibiotics was the critical threat in cases of destroying ecological balance among aquatic organisms, and contributing undesirable antibiotic resistant bacteria (ARB) into the environment [5]. The majority of antibiotics (approximately 90%) were excreted from humans and animals through their urine and feces after administration and it significantly passed through the terrestrial and aquatic environment. As Halling-Sorensen and other [6] noticed that the concentration of antibiotics in surface and groundwater increased to lg/L and ng/L range, respectively. The presence of antibiotic pollutant in waste stream creates a selective pressure causing mutant microorganisms and contributing to proliferating antibiotic resistance microorganisms [7]. Many of these antibiotic contaminants found in natural surface water and sewage treatment plant effluents were derived from those that were originally designed to control bacteria in humans and animals [8-10]. Consistent traces of antibiotics at concentrations less than 1 μg/L have been detected in the samples of their environmental medium [11]. ARB and the occurrence of antibiotic resistance genes in many sewage treatment facilities have drawn a lot of public concerns and been well linked to their adverse impacts on natural aquatic systems [12]. Such increasing potential risks of antibiotic-resistant bacteria and tolerance of antibiotics by human and livestock are predominantly documented [13].

The antibiotics-contaminated was necessary to be treated adequate to make effluent free from active antibiotics before releasing into aquatic body. It has been well acknowledged that conventional wastewater treatment plants are not effective in removing pharmaceutical pollutants since they are not designed for such matters. However the method is very expensive, requiring a high level of technical knowhow and well trained treatment plant operators, a steady energy supply, and chemicals and specific equipment which may not be readily available. Chlorination remains the most common form of wastewater disinfection due to its low cost and long-term history of effectiveness. One disadvantage is that chlorination of residual organic material can generate chlorinated-organic compounds that may be carcinogenic or harmful to the environment. Residual

chlorine or chloramines (formed by the combination of chlorine and ammonia) may also be capable of chlorinating organic material in the natural aquatic environment. Further, because residual chlorine is toxic to aquatic species, the treated effluent must also be chemically dechlorinated adding to the complexity and cost of treatment [14]. Ultraviolet (UV) light can be used instead of chlorine. Because no chemicals are used, the treated water has no adverse effect on organisms that later consume it. UV radiation causes damage to the genetic structure of bacteria, viruses, and other pathogens making them incapable of reproduction. The key disadvantages of UV disinfection are the need for frequent lamp maintenance and replacement, and the need for a highly treated effluent to ensure that the target microorganisms are not shielded from the UV radiation. [15].

There is a need to develop effective technology and treatment methods for the degradation of antibiotic pollutants, either to less harmful compounds or to their complete mineralization. The promising advanced oxidation processes (AOPs) have been widely used for wastewater treatment to removal organic and inorganic contaminants from drinking water and industrial wastewater [16]. The action of AOPs on the active substances was based on the reaction of highly free radicals, like hydroxyl radical ($HO^{\bullet}$), which was produced via chemical (O_3/OH^-, O_3/H_2O_2, Fe^{2+}/H_2O_2), photochemical (UV-C/H_2O_2, UV-C/O^3) and photocatalytic reactions (UV-A/TiO^2) [17]. Various studies have been shown the effectiveness of different AOPs methods for degradation of various antibiotics compounds, for example sulfonamide and macrolide antibiotics [18-19]. With these developed AOP methods, the antibiotics substances can be completely removed in the short time from 10 min to longer than 1 h depending on types of antibiotics. Among the most common chemical substances used for reactant $HO^{\bullet}$, H_2O_2 can potentially generate a high oxidation potential value after ozone [20] but its cost was the most economical. None has not been studied the individual H_2O_2 effect and H_2O_2 with elevated temperature for the removal of pharmaceutical antibiotics. In this research, the use of H_2O_2 combined with various elevated temperature levels was evaluated for the inactivation of three antibiotics (ceftazidime, ceftriazone, and cefphalexinantibiotics) in the synthetic antibiotic formulation wastewater. Three environmentally relevant pharmaceuticals were chosen according to the routine productions and local consumptions from our collaborative pharmaceutical.

Objectives

This research focuses on the elimination of antibiotic contaminated in pharmaceutical wastewater by using AOP application at various temperatures. Simulated antibiotic wastewater at 60 μg/mL concentration represents average loads of 3 antibiotics (i.e., ceftazidime, ceftriaxone and cephalexin). Also H_2O_2 treatments at 1 3 and 5% (v/v) were applied and the treatment temperatures were varied from 60 to 150°C. The antibiotic residue after treatment was evaluated using the microbiological test and compared to determine the optimal conditions for antibiotic wastewater treatment.

Methods

Reagents and chemicals; ceftazidime, ceftriaxone and cephalexin antibiotics were received from Milimed Co., Ltd., at the highest available purity (>98%). Individual stock standard solutions of the antibiotics were prepared in deionized (DI) water on a weight basis at concentration 60 μg/mL. Standard mixtures were

prepared by dilution of the stock solutions before each analytical run. H_2O_2 (50%) v/v was supplied by Chemipan Corporation Co., Ltd.

Antibiotic wastewater models; Industrial wastewater samples were collected from the first wash of processing equipment after the production. The wastewater samples were collected in amber bottles used within 2 h following sampling. The averaged concentration of each antibiotic wastewater sample was determined using HPLC protocol to formulate the concentration of the model wastewater.

Reactor setup and H_2O_2 treatment; The H_2O_2 treatment was perform on the model antibiotic wastewater in an isothermal reactor (Figure 1). The stock standard of the antibiotic cocktail was prepared at 1,200 μg/mL. Ten-fold dilution was obtained prior to treatment. Two-fold H_2O_2 solution was used to mix with the same portion of the diluted stock standard to achieve 100 mL reaction volume. The antibiotic and H_2O_2 mixture was poured into the reactor vessel and incubated at 60 80 and 100°C in an hot oil bath. The temperature of the mixture was monitored using a type-K thermocouple connected to an elecctronic temperature reader. At each sample interval, 5 mL sample were drawn from the reactor for further analyses.

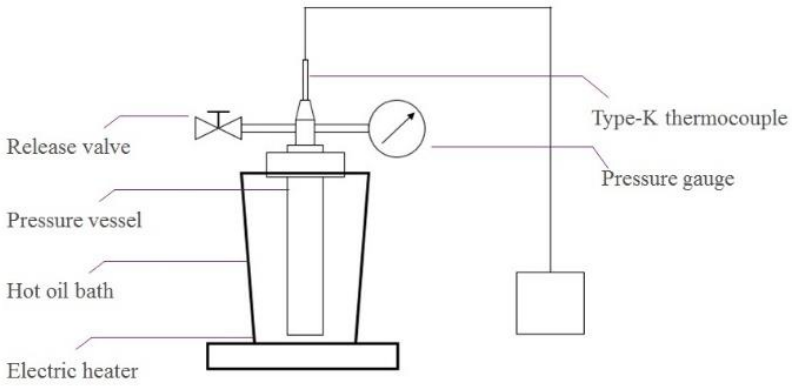


Figure 1 Reactor Setup

Neutralization of H_2O_2 by dried yeast powder; H_2O_2 solutions were prepared in DI water at 1 3 and 5% (w/v) and 50 mL of H_2O_2 solution were mixed with dried yeast powder in a beaker to catalyze oxygen and water conversions at the ratio of yeast to H_2O_2 solution (1:50). The mixture was constantly stirred at room temperature. Samples were drawn intermittently within 30 min and analyzed for *E. coli* count and residual H_2O_2 concentration.

Determination of antibiotic residues after treatment by HPLC analysis. The amount of residual antibiotics were determined with a HPLC equipped with a C8 reversed phase liquid chromatography (LC) column (4.6 x 150 mm) at a flow rate of 1 mL/min, 30°C using solvent system of methanol: 40 mM KH_2PO_4 (18:82). Absorbance of the eluent was monitor at 254 nm. Injection volume was at 10 μL.

Microbial evaluation; The antibiotic residue after treatment was evaluated using the microbiological test and compared to determine the optimal conditions for antibiotic wastewater treatment by measuring the *E. coli* growth in synthetic wastewater. The number of viable cells was determined by a viable count method. To perform *E. coli* colony count, a minaturized innoculation protocol was obatined from Sangadkit and other

[21]. Ten- fold dilution was prepared in normal saline water and used as a spiked *E. coli* cells to be inoculated into the treated antibiotic wastewater model. The final cell count after inoculation was compared to the control sample to evaluate the remaining antibiotic activity. 10 µL of the serially-diluted samples were inoculated into the 96-microtiter plate containing 500 µL of Chromocult® Coliform Agar (CCA). Then the microtiter plates were incubated at 37^{O}C for 12 h. Enumeration of *E. coli* colonies were reported as CFU/mL.

Results

Simulation of antibiotic wastewater model

Occurrence of antibiotics in the first wash of equipment cleaning was analyzed and the HPLC chromatogram showed ceftriaxone was present at an average of 10 µg/mL (Figure 2).

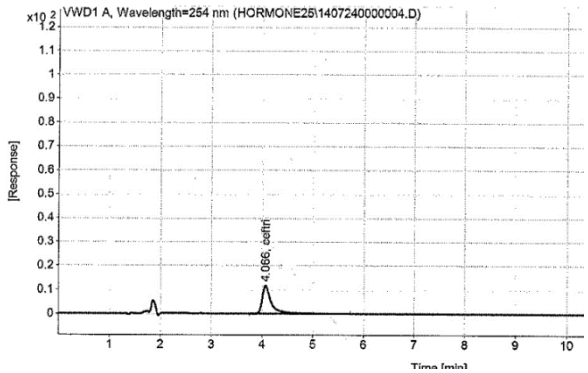


Figure 2 HPLC chromatogram of typical ceftriaxone wastewater (approximately 10 µg/mL)

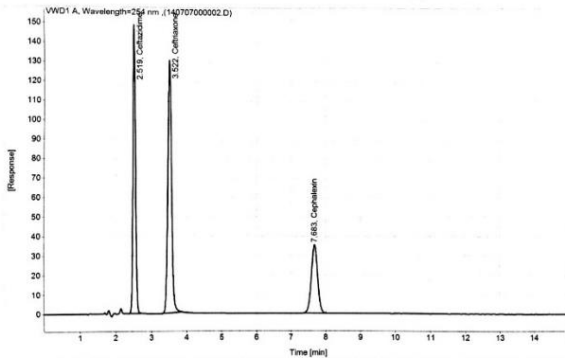


Figure 3 HPLC chromatograms showing well-separated peaks of ceftazidime, ceftriaxone and cephalexin of the model antibiotic wastewater at the concentration of 60 µg/mL each

To accommodate HPLC analysis, six-fold increase of the original wastewater concentration was applied to construct a model wastewater to perform H_2O_2 treatment. At the same antibiotic production facility, there are two other antibiotics (ceftazidime and cephalexin) produced; hence, these antibiotics were included to form a wastewater cocktail. Figure 3 illustrated the well-distributed antibiotic peaks in the same HPLC chromatogram of a model wastewater containing six-fold strength of the averaged concentration (60 µg/mL each) or "a 1X wastewater model". The resulting chromatogram of the formulated model wastewater displayed well-distributed peaks of each antibiotic having different retention times at 2.51, 3.52, and 7.68 for ceftazidime, ceftriaxone and cephalexin, respectively.

H_2O_2 neutralization using dried baker yeast treatment

Prior to perform microbiological analysis of the treated antibiotic wastewater, the residual H_2O_2 in the wastewater solution is to be neutralized to remove H_2O_2 toxicity. The H_2O_2 detoxification was achieved by using enzymes, called peroxiredoxins, which are abundant in yeasts [22]. An experiment was setup to demonstrate the reduction of H_2O_2 to oxygen and water through the use of reducing equivalents provided by thioredoxin and other thiol-electron donors [23]. Figure 4 illustrated the reduction of H_2O_2 residues in the mixture of 1 3 and 5% H_2O_2 and baker's yeast powder at 1:50 (powder to solution ratio). In this semi-log plot, the yeast powder catalyzed self-decomposition of H_2O_2 to water and oxygen following first-order degradation kinetics and having approximately the same rate of conversion (0.14-0.15 min^{-1}).

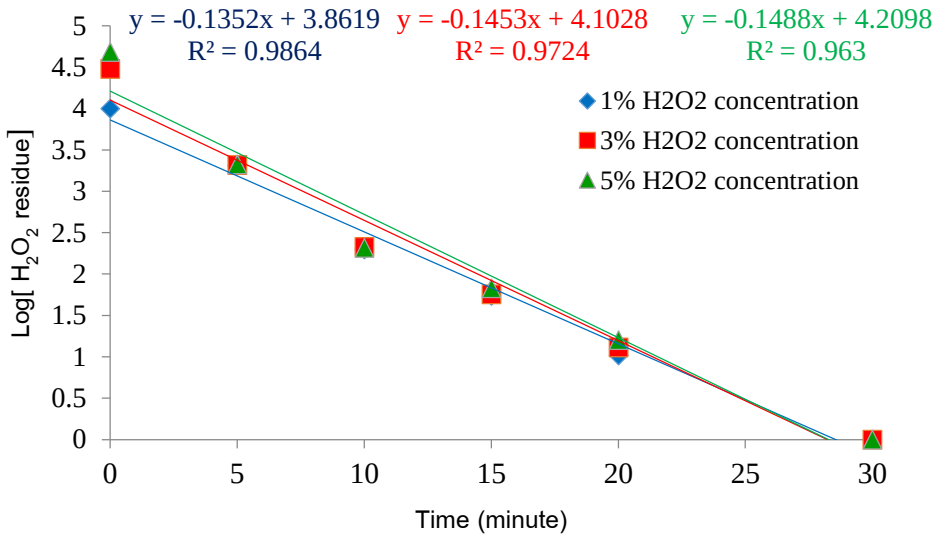


Figure 4 Residual H_2O_2 concentrations at different incubation times after treated with baker's yeast per at 1:50 powder to solution ratio

To evaluate the remaining toxicity of the residual H_2O_2 in the mixture solution, *E. coli* culture at log 6 CFU/mL initial density was introduced into the solution with one tenth dilution to reach the final cell concentration at log 5 CFU/ml in the H_2O_2 solution. The colony count was performed on the yeast powder addition experiment against the control experiment without yeast powder. In Figure 5, the control without yeast addition showed no *E. coli* colony in all H_2O_2 solutions. After 5 min incubation in yeast powder mixture, the level of residual H_2O_2 dropped substantially (Figure 4) and the colony count result showed much improved *E. coli* cell recovery closed to the expected cell density of log 5 CFU/ml in all mixtures of different H_2O_2 concentrations.

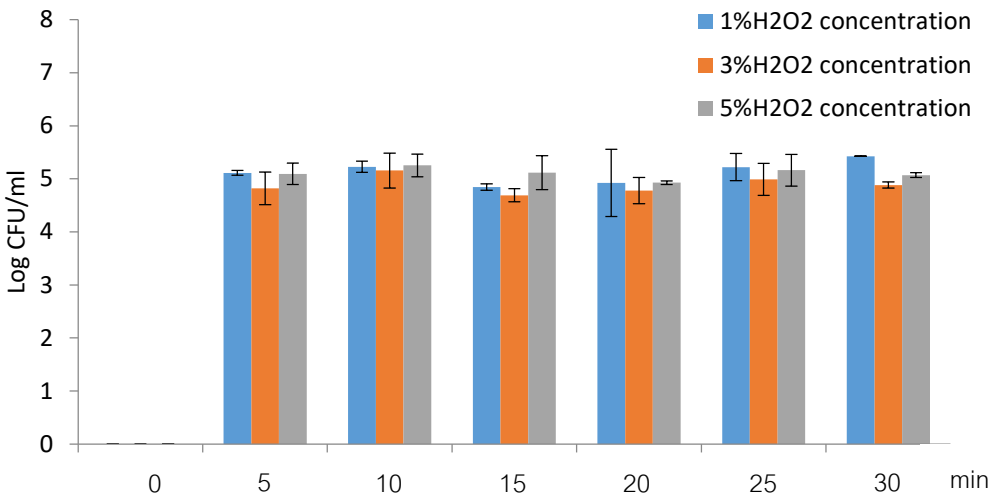


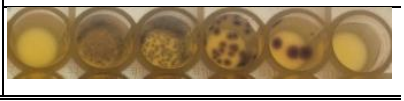
Figure 5 *E. coli* colony count at different incubation times showing the effect of peroxidase activity from dried baker's yeast powder on H_2O_2 conversion

In Figure 5, the result of *E. coli* colony count at different incubation times showed the inhibitory effect of H_2O_2 was largely minimized when yeast powder was added. The 1:50 ratio of yeast powder to H_2O_2 solution was sufficient to neutralize H_2O_2 toxicity up to 5% H_2O_2 solution. To take into account the concentration of residual H_2O_2 concentration in Figure 5, the 30 min incubation time was selected to eliminate the possibility of H_2O_2 inhibition in the subsequent experiments.

Degradation of antibiotics in contaminated wastewater by H_2O_2 treatment.

In the control experiment where no trace of antibiotic present, the introduction of *E. coli* showed the final concentration of log 5 CFU/mL and *E. coli* colonies grew well in serial dilution order in all agar culture wells (Table 1).

Table 1 The effect of temperature and incubation time of 1%H_2O_2 treatment on degradation of antibiotic and *E. coli* count. (N/A = not applicable)

Initial antibiotic concentration (μg/mL)	Treated temperature (^{O}C)	Incubation time (min)	Residual antibiotic after treated (μg/mL)			*E. coli* count (CFU)					
			Ceftazidime	Ceftriaxone	Cephalexin	10-fold serial dilution					
						0	-1	-2	-3	-4	-5
0 (control)	27	N/A	0	0	0						
60	60	1	43.27	55.11	44.31						
		3	37.78	45.80	37.79						
		5	34.74	41.61	34.52						
	80	1	46.10	30.91	34.68						
		3	20.14	3.91	18.15						
		5	12.78	1.152	11.56						
	100	1	15.74	2.56	1.75						
		3	2.56	2.52	2.16						
		5	0.06	2.43	0						

When *E. coli* was cultured using the treated wastewater containing residual antibiotics, the biostatic effect of tested antibiotics showed no growth or very few colony formation in the wells with low and no dilutions. The degree of antibiotic degradation was increased with higher temperatures and longer incubation times. The result seemed to indicate that the order of bio-recalcitrance was as follows: ceftazidime > ceftriaxone > cephalexin. There was a study also showed different removal efficiency of different classes of antibiotics (tetracyclines > sulfonamides > quinolones) in a conventional sewage treatment plant (STP) enabling an average of 87.9% tetracyclines removal from this experiment [12]. Our 1% H_2O_2 treatment can degrade 86.23% of ceftazidime with 5 min incubation at 100^{O}C.

Effect of H_2O_2 concentration on antibiotic degradation

Higher concentration of H_2O_2 was able to degrade more antibiotics to achieve lesser final residual concentrations. The residual antibiotics were generally less at 5% H_2O_2 treatment than that at the 3% H_2O_2 treatment (Table 2 and 3).

Table 2 The effect of temperature and incubation time of 3% H_2O_2 treatment on degradation of antibiotic and *E. coli* count. (N/A = not applicable)

Initial antibiotic concentration (μg/mL)	Treated temperature (^{O}C)	Incubation time (min)	Residual antibiotic after treated (μg/mL)			*E. coli* count (CFU)
			Ceftazidime	Ceftriaxone	Cephalexin	10-fold serial dilution
0 (control)	27	N/A	0	0	0	
60	60	1	40.40	41.55	35.15	
		3	26.81	38.07	25.76	
		5	25.93	32.27	22.93	
	80	1	30.18	34.21	32.93	
		3	22.57	9.74	24.97	
		5	18.91	0	0	
	100	1	31.95	17.41	31.90	
		3	16.28	9.91	0	
		5	8.26	0	0	

0 -1 -2 -3 -4 -5

Table 3: The effect of temperature and incubation time of 5% H_2O_2 treatment on degradation of antibiotic and *E. coli* count. (N/A = not applicable)

Initial antibiotic concentration (µg/mL)	Treated temperature (^{O}C)	Incubation time (min)	Residual antibiotic after treated (µg/mL)			*E. coli* count (CFU)
			Ceftazidime	Ceftriaxone	Cephalexin	10-fold dilution: 0 / -1 / -2 / -3 / -4 / -5
0 (control)	27	N/A	0	0	0	
60	60	1	50.00	40.62	42.18	
		3	44.78	30.51	35.03	
		5	35.32	17.55	22.56	
	80	1	6.35	2.15	0	
		3	3.67	1.41	0	
		5	2.02	1.74	0	
	100	1	5.10	0	0	
		3	2.72	0	0	
		5	1.40	0	0	

Extended H_2O_2 treatment at 100^{O}C

In this experiment, the incubation period was shifted from the course of maximum 5 min to 2 h time frame. When incubation time was extended, the long exposure to oxidation condition even at 1% H_2O_2 treatment provide higher degree of antibiotic degradation (Table 4). At 10 min, both ceftriaxone and cephalexin were eliminated. With the 1% H_2O_2 treatment, the ceftazidime experiment required up to 2 h to achieve total removal from the wastewater. When the H_2O_2 concentration was increased to 3 and 5%, the incubation times were reduced to 60 and 30 min, respectively.

Table 4: The effect of heated temperature at 100°C and incubation time of 1 3 and 5%H_2O_2 treatment on degradation of antibiotic and *E. coli* count.

Initial antibiotic concentration (µg/mL)	Time (min)	H_2O_2 Concentration (%)	Residual antibiotic after treated (µg/mL)			*E. coli* count (CFU)					
			Ceftazidime	Ceftriaxone	Cephalexin	10-fold dilution					
						0	-1	-2	-3	-4	-5
0 (Control)	0	N/A	0	0	0						
60	10	1	4.314	0	0						
	30		0.894	0	0						
	60		0.834	0	0						
	120		0	0	0						
	10	3	3.45	0	0						
	30		0.426	0	0						
	60		0	0	0						
	120		0	0	0						
	10	5	2.232	0	0						
	30		0	0	0						
	60		0	0	0						
	120		0	0	0						

Conclusions and Discussion

As the incubation time increased to 30 min, the *E. coli* colony count was fairly constant suggesting the H_2O_2 concentration was sufficiently low and *E. coli* cells to grow without inhibitory effect of H_2O_2. This H_2O_2 neutralization method by adding yeast powder worked well and enabled the destruction of H_2O_2 so that only the inhibitory effect of remaining antibiotics was explored.

In Table 1 the biocidal effect of antibiotics was subsided as the solution was more diluted. There was less likelihood of colony formation on the cultured agar at the -5 dilution indicating lingering biocidal effect of this untreated antibiotic solution. The HPLC chromatogram results (shown elsewhere) suggested the 1% H_2O_2 treatment was adequate to cause the degradation of the antibiotic activity in all types and concentrations. The

results of the residual antibiotic from different treatments of temperature and incubation time suggested different resiliency of different antibiotic species. The degradation of antibiotic activity can be best explained by similar results of AOP applications in water and wastewater treatments. The oxidizing capability of hydroxyl radicals (•OH) generated by AOPs has been demonstrated to remove natural organic matters in the raw water via chemical oxidation [24]. Degradation mechanism was based on the chemistry of •OH, which are non-selective reactive species enabling oxidization of pollutants into mineral end-products and yielding CO_2 and inorganic ions [25]. In an UV- and O_3-based AOPs, an improved efficiency of phenol degradation by increasing H_2O_2 concentration was observed and the addition H_2O_2 was able to enhance mineralization of phenol in model wastewater [26]. UV/H_2O_2 treatment was also used to minimize organic pollutants and antibiotic in aqueous solution and tested on wastewater matrices containing 40 selected pharmaceuticals and showed that these compounds were able to be directly degraded by H_2O_2 even without UV irradiation [27]. In this study, some pharmaceuticals tramadol, terbutaline and penicillin V were disappeared after addition of H_2O_2 in the water samples whereas other compounds, for example pindolol, sotalol, salbutamol, lincomycin and clindamycin showed much lower concentrations than initially concentrations.

As seen before, the higher temperature treatment was able to generate stronger oxidation condition, presumably more •OH generated. At the 100°C treatment, both ceftriaxone and cephalexin were totally removed from the model wastewater and only trace of ceftazidime was detected. The evaluation of antibiotic toxicity to *E. coli* cells agreed well with the HPLC results. The CCA wells with no dilution showed some indication of *E. coli* growth at higher treated temperature and long incubation time, similar to that of the control condition (Tables 2 and 3). All treatments (at 5% H_2O_2 and 100°C) showed good colony formation in all dilutions even at the no dilution treatment. All results indicated neither biostatic nor biocidal effects of the antibiotics comparing to those with high concentrations of residual antibiotics. There was a study successfully demonstrating the use of low H_2O_2 dosages to treat antibiotics effluent via UV/H_2O_2 processes. However, low concentrations of H_2O_2 decreased biodegradability or mineralization of the organic matters. Higher H_2O_2, on the other hand, was able to mineralize biocompatible compound in wastewater effluents. One way to increase •OH oxidation at low H_2O_2 treatment was to extend the incubation time and allow longer •OH mediated advanced oxidation process.

In Table 4 with longer incubation period, it was possible to oxidize ceftazidime completely for the first time as opposed to shorter incubation times in the previous experiments. It was evident that ceftazidime was the most recalcitrant among the three tested antibiotics. All the *E. coli* count experiments indicated that residual antibiotics were very low and did not affect the growth of *E. coli* in all dilutions.

The removal of antibiotics from the model wastewater using H_2O_2 treatment at elevated temperatures was demonstrated. The application of high H_2O_2 concentrations and temperatures was able to accelerate the biodegradability of all three antibiotics. Ceftriaxone and cephalexin were more susceptible to the H_2O_2 treatment, especially at high temperatures, than ceftazidime. Prolong treatment of H_2O_2 at these low concentrations was able to achieve total removal of all three antibiotic. At 100°C, the shortest time to achieve no detection of all three antibiotics was 30 min using 5% H_2O_2. Longer incubation was needed for lower H_2O_2 concentrations. *E. coli* cell count results agreed well with the HPLC analysis. The use of yeast powder was able to neutralize

the H_2O_2 toxicity and allowed the effect of biostatic and biocidal effects of the three antibiotics to be thoroughly scrutinized in this study.

The results from this study showed a great potential of oxidation using H_2O_2 at high temperature to treat pharmaceutical wastewater. This technology was shown to have a capable degrade 3 artificial wastewaters from our collaborative pharmaceutical. This laboratory-scale experiments could be upgraded to a pilot- and industrial-scale equipment with simple engineering. More studies should be done to realize this effective wastewater treatment process for pharmaceutical wastewater treatment and a larger prototype equipment should be constructed to test the concept in a larger scale.

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